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(54) **Feeds containing bacillus subtilis C-3102.**

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Description

The invention relates to Bacillus subtilis C-3102 and its use.

Bacillus subtilis C-3102 in accordance with the present invention exhibits remarkable effects in increasing the weight and in improving the feed efficiency, when it is given to animals, such as mammals, fowls, and fishes as a foodstuff. This is important and advantageous in the area of livestock industry and fisheries since animal meat is very important as a foodstuff and as feed. Therefore it is strongly desired in the industrial world to efficiently raise economic animals. Extensive investigations have been made in the past to find a formulation ratio of main ingredients which are inexpensive and have good nutrition balance, vitamins and minerals as trace components, and the like. Further a variety of probiotics (live bacterial agents) have been tested and the effects thereof have been checked; but the results are yet unsatisfactory since usefulness and compatibility can be considered always within only small ranges of animals; and e.g. GB-A-10 69 400 of 1965 mentions an animal feed supplement made in a special cultivating mode of a microorganism of the species Bacillus subtilis N' or Bacillus natto; another example of 1937 is US-A-21 32 621 which mentions a feed composition for animals where soya meal is used containing microorganism strains, e.g. B. mesentericus; a third example where specific kinds of microorganisms, such as B. natto, is used is given in JP-A-59 128 334.

Thus the present problem is the actual situation that satisfactory probiotics having excellent safety and also weight increasing effect are necessary in the feed industry. Consequently the object of the present invention is directed to this aim.

According to the invention this object has been solved by the probiotic for animals according to claim 1 which comprises Bacillus subtilis C-3102 (FERM BP-1096); such bacteria have been neither found heretofore nor has it been known that this new strain shows excellent effects as probiotic.

It has been found that when this Bacillus subtilis C-3102 is incorporated and contained in animal feed it can accelerate the gain of weight in animals and at the time can improve the feed efficiency. Thus the present claim 2 describes the use of Bacillus subtilis C-3102 as a probiotic for animals and claim 3 relates to feed containing Bacillus subtilis C-3102.

The strain as used, i.e. Bacillus subtilis C-3102, has been newly isolated from the natural world in conjunction with the present invention.

Bacillus subtilis C-3102 has been deposited in Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, under FERM BP-1096. Bacteriological properties of this strain are as follows:....

A. Morphological properties
   (1) Cells: rods having a width of 0.6 to 1.0 μm and a length of 1.5 to 2.0 μm.
   (2) Gram-positive
   (3) Spores: oval
   (4) Mobility: positive
   (5) Aerobic

B. Growth Conditions in Various Media
   (1) Nutrient agar: excellent
   (2) Anaerobic glucose bouillon: -
   (3) 7 % NaCl: +

C. Physiological Properties
   (1) Growth at 50 °C: +
   (2) Growth at pH 5.7: +
   (3) Utilization of citrate: +
   (4) Acid production from carbohydrates arabinose, glucose, xylose, mannitol: +
   (5) VP reaction: +
   (6) Hydrolysis of starch: +
   (7) Reduction of nitrate: +
   (8) Production of indole: -
   (9) Liquefaction of gelatin: +
   (10) Decomposition of casein: +
   (11) Film formation on liquid medium: +
   (12) Coagulation of milk: -
   (13) Peptonization of milk: +
   (14) Catalase: +

Next, a method for culturing Bacillus subtilis C-3102 of the present invention will be described.

As media, there can be widely used media ordinarily used for culturing microorganisms containing carbon sources, nitrogen sources, inorganic matters, vitamins, amino acids, etc. The carbon source can be any carbon compounds which are assimilable and, for example, glucose, sucrose, starch, molasses, etc. can be employed. The nitrogen source can be any nitrogen compounds which are utilizable and, for example, peptone, meat extract, casein acid hydrolysate, ammonium sulfate, etc. can be employed. In addition, phosphates, salts of metals such as magnesium, sodium, potassium, calcium, iron, manganese, etc.; vitamins, amino acids, defoaming agents, surfactants, etc. can also be used, depending upon necessity.

As the media, both liquid media and solid media can be used and incubation under aerobic conditions is appropriate.

Incubation can be suitably effected at an initial pH of the medium is pH of 5 to 9, preferably 6 to 8, at culturing temperatures at 20 to 50 °C, preferably 35 to 40 °C and for a culturing time period of 12 hours to 7 days.

The thus obtained culture can be used as it is or as washed bacteria. Further the culture or bacteria can also be used as it is or after additives (carries innoxious to animals) are added thereto followed by drying or preparations thereof are produced. The probiotics of the present invention include these forms for use, which comprises Bacillus subtilis C-3102. It is sufficient that Bacillus subtilis C-3102 be incorporated in an amount of 10 to 10^{12} cells, preferably 10^4 to 10^8 cells, per 1 g of a feed but the amount is not limited only to such a range.

When the feed containing this bacteria is given to animals, a great weight-increasing effect can be obtained and feed conversion can also be greatly improved.

Moreover, the feed containing the bacteria is extremely effective for a variety of animals, in addition to mammals such as cows, pigs, horses, sheep, etc., various fowls such as chicken, quails, etc., and fishes. Furthemore, the feed is also useful for various pet animals such as dogs, cats, goldfishes, canaries, etc.

Hereafter preparation examples of the bacteria and reference examples showing fatting tests using the obtained probiotic will be described in detail.

Preparation Example 1

Medium obtained by dissolving 400 g of soybean peptone, 10 g of dibasic potassium phosphate and 200 g of molasses in 60 liters of tap water and adjusting pH to 7.5 with 1 N sodium hydroxide solution was charged in a jar fermenter and sterilized at 121 °C for 15 minutes. A culture solution of Bacillus subtilis C-3102, FERM BP-1096, which had been previously subjected to preliminary incubation, was inoculated thereinto followed by spinner culture under aeration at 37 °C for 40 hours.

The thus obtained culture solution was centrifuged to collect the cells. After drying, the cells were incorporated in skim milk powder to give 10 kg of a probiotic of Bacillus subtilis C-3102. The viable count contained in this probiotic was 1×10^9 cells/g.

Preparation Example 2

To 5 kg of soybean oil meal granules (Fujinic Ace 500, manufactured by Fuji Purina Protein Co., Ltd.) was added 5 kg of tap water followed by sterilization at 121 °C for 120 minutes. A culture solution of Bacillus subtilis C-3102, FERM BP-1096, which had been previously subjected to preliminary incubation, was inoculated thereinto followed by culture at 37 °C for 40 hours.

The thus obtained culture solution was dried and reduced into powder. The powder was incorporated in calcium carbonate to give 4 kg of a probiotic of Bacillus subtilis C-3102. The viable count contained in this probiotic was 1×10^{10} cells/g.

Example 1

Male ICR mice of 6 age in weeks were divided into 2 groups (test gr. and control gr.) of 10 each and the feeds having the compositions as shown in Table 1 were given to the respective groups to conduct growth test. The probiotic obtained in Preparation Example 1 by incorporating into skim milk powder was used. The viable count in the feed in which the probiotic was added was 1×10^6 cells/g. The results are as shown in Table 2.

Table 1 (unit: wt%)

	Invention	Control
CE-2(feed for mouse, manufactured by CLEA JAPAN, INC.	99.9	99.9
Skim milk powder	-	0.1
Probiotic of the present invention	0.1	-

Table 2

	Invention	Control
Feeding period (days)	19	19
Mean body weight at start(g)	29.0	29.0
Mean body weight at end(g)	35.2	33.6
Mean body weight gain(g)	6.2	4.6
[Body weight gain index]	[135]	[100]
Mean feed intake(g)	109.2	103.0
Feed conversion ratio	17.6	22.4

As shown in Table 2, it is understood that by feeding the formula feed added with the probiotic of the present invention, an outstanding body weight-increasing effect was obtained and at the same time, the feed conversion ratio was also improved.

Example 2

Piglings of 3 to 4 age in weeks were divided into 2 groups (test gr. and control gr.) of 6 each and the feeds having the compositions as shown in Table 3 were given to the respective groups to conduct growth test. The probiotic obtained in Preparation Example 2 by incorporating into calcium carbonate was used. The viable count in the feed in which the probiotic was added was 1×10^6 cells/g. The resuls are as shown in Table 4.

Table 3 (unit: wt%)

	Invention	Control
Bakery waste (PANBIS, manufactured by Koike-shoten Co.)	37	37
Skim milk powder	35.99	35.99
Feed which has adsorbed soybean extract (BITAZE 8, manufactured by Enaze Sangyo Co., Ltd.)	11	11
Glucose	6	6
Dehulled soybean powder (PAFESU 109, manufactured by Kikkoman Co., Ltd.)	3	3
Vitamins, minerals and other additives	7	7
Calcium carbonate	-	0.01
Probiotic of the present invention	0.01	-

Table 4

	Invention	Control
Feeding period (days)	21	21
Mean body weight at start(kg)	6.1	6.1
Mean body weight at end (kg)	13.9	13.2
Mean body weight gain(kg)	7.8	7.1
[Body weight gain index]	[110]	[100]
Mean feed intake (kg)	67.9	67.3
Feed conversion ratio	1.45	1.58
Observation of feces (points: cf. Note)	19	54

Note: Total points of each group when normal feces, soft feces, diarrhea and watery feces were designated 0, 1, 2 and 3 points, respectively.

As shown in Table 4, it is understood that by feeding the formula feed added with the probiotic of the present invention, an outstanding body weight-increasing effect was obtained and at the same time, the feed conversion ratio was also improved, and further that the occurrence of diarrhea and soft feces was greatly reduced.

Example 3

Broilers of 2 to 3 age in days were divided into 2 groups (test gr. and control gr.) of 30 each (15 females and 15 males) and the feeds having the compositions as shown in Table 5 were given to the respective groups to conduct growth test.

The probiotic obtained in Preparation Example 1 by incorporating into skim milk powder was used. The viable count in the feed in which the probiotic was added was 1 x 10^6 cells/g. The results are as shown in Table 6.

Table 5

	(unit: wt%)	
	Invention	Control
Commercial formula feeds for broilers for use in a former feeding period (20 days) and for use in a latter feeding period (36 days) (manufactured by Kumiai Feed Co.)	99.9	99.9
Skim milk powder	-	0.1
Probiotic of the present invention	0.1	-

Table 6

	Invention	Control
Feeding period (days)	56	56
Mean body weight at start(g)	40	40
Mean body weight at end(g)	2150	2000
Mean body weight gain(g)	2110	1960
[Body weight gain index]	[108]	[100]
Mean feed intake (g)	4642	4528
Feed conversion ratio	2.20	2.31

As shown in Table 6, it is understood that by feeding the formula feed added with the probiotic of the present invention, an outstanding body weight-increasing effect was obtained and at the same time, the feed conversion ratio was also improved.

Example 4

Chicken for harvesting eggs were divided into 2 groups (test gr. and control gr.) of 30 each and the feeds having the compositions shown in Table 7 were given to the respective groups to conduct a test. The probiotic obtained in Preparation Example 1 by incorporating into skim milk powder was used. The viable count in the feed in which the probiotic was added was 1×10^6 cells/g. The results are as shown in Table 8.

Table 7 (unit: wt%)

	Invention	Control
Commercial formula feed (Kumiai Feed Co.)	99.9	99.9
Skim milk powder	-	0.1
Probiotic of the present invention	0.1	-

Table 8

	Invention	Control
Feeding period (days)	28	28
Total number of laid eggs	712	672
[Laid-egg number index]	[106]	[100]
Mean egg weight (g)	60.2	59.2
[Egg weight index]	[102]	[100]

As shown in Table 8, it is understood that by feeding the formula feed added with the probiotic of the present invention, the number of laid eggs increased and at the same time, the mean egg weight also

increased.

Example 5

Rainbow trout fry of approximately 14 g were divided into 2 groups (test gr. and control gr.) of 30 each and the feeds having the compositions as shown in Table 9 were given to the respective groups to conduct growth test. The probiotic obtained in Preparation Example 1 by incorporating into skim milk powder was used. The viable count in the feed in which the probiotic was added was 1×10^6 cells/g. The results are as shown in Table 10.

Table 9 (unit: wt%)

	Invention	Control
Northern-sea fish meal	55	55
α-Starch	20	20
Dextrin	5	5
Oil mixture	10	10
Cellulose	3	3
Vitamins, minerals and other additives	6.9	6.9
Skim milk powder	-	0.1
Probiotic of the present invention	0.1	-

Table 10

	Invention	Control
Feeding period (days)	45	45
Mean body weight at start(g)	14.3	14.0
Mean body weight at end (g)	39.6	37.6
Mean body weight gain(g)	25.3	23.6
[Body weight gain index]	[107]	[100]

As shown in Table 10, by feeding the formula feed added with the probiotic of the present invention, an outstanding body weight-increasing effect can be noted.

Example 6

Calves of 1 week age after birth were divided into 2 groups (test gr. and control gr.) of 4 each and the feeds having the compositions as shown in Table 11 were given to the respective groups to conduct growth test. The probiotic obtained in Preparation Example 2 by incorporating into calcium carbonate was used. The viable count in the feed in which the probiotic was added was 1 x 10^6 cells/g. Upon peforming the test, 1 part by weight of the feed was dissolved and dispersed in 7 parts by weight of hot water, which was then fed to the calves. The results are as shown in Table 12.

Table 11

	(unit: wt%)	
	Invention	Control
Skim milk powder	70	70
Whey	10	10
Beef tallow	12.49	12.49
Lecithin	0.5	0.5
Vitamins, minerals and other additives	7	7
Calcium carbonate	-	0.01
Probiotic of the present invention	0.01	-

Table 12

	Invention	Control
Feeding period (days)	28	28
Mean body weight at start(kg)	43.7	43.9
Mean body weight at end(kg)	75.3	73.9
Mean body weigt gain (kg)	31.6	30
[Body weight gain index]	[105]	[100]
Mean feed intake (kg)	44.9	44.1
Feed conversion ratio	1.42	1.47
Observation of feces (points: cf. Note)	29	45
Note: Total points of each group when normal feces, soft feces, diarrhea and watery feces were designated 0, 1, 2 and 3 points, respectively.		

As shown in Table 12, it is understood that by feeding the formula feed added with the probiotic of the present invention, an outstanding body weight-increasing effect was obtained and at the same time, the feed conversion ratio was also improved, and further that the occurrence of diarrhea and soft feces was greatly reduced.

Claims

1. Probiotic for animals which comprises Bacillus subtilis C-3102 (FERM BP-1096) having bacteriological properties described below:
   (1) Gram-positive,
   (2) Cells: rods,
   (3) Spores: oval,
   (4) Mobility: positive,
   (5) Aerobic,
   (6) Catalase: positive,

(7) Growth at 50 °C: +,
(8) Growth at pH 5.7: +,
(9) Utilization of citrate: +,
(10) Acid prodution from saccharides:
arabinose, glucose, xylose, mannitol: +,
(11) VP reaction: +,
(12) Hydrolysis of starch: +,
(13) Reduction of nitrate: +,
(14) Production of indole: -,
(15) Liquefaction of gelatin: +,
(16) Decomposition of casein: +,
(17) Film formation on liquid medium: +,
(18) Coagulation of milk: -, and
(19) Peptonization of milk: +,

2. Use of Bacillus subtilis C-3102 (FERM BP-1096) as a probiotic for animals.

3. Feed containing Bacillus subtilis C-3102(FERM BP-1096) as defined in claim 1.

Patentansprüche

1. Probiotikum für Tiere, welches den Bacillus subtilis C-3102 (FERM BP-1096) umfaßt, der die unten beschriebenen bakteriologischen Eigenschaften aufweist:
(1) Gram-positiv
(2) Zellen: Stäbchen
(3) Sporen: oval
(4) Beweglichkeit: positiv
(5) Aerob
(6) Catalase: positiv
(7) Wachstum bei 50 °C: +
(8) Wachstum bei pH 5,7: +
(9) Verwendung von Citrat: +
(10) Säureproduktion aus Sacchariden:
Arabinose, Glucose, Xylose, Mannit: +
(11) VP-Reaktion: +
(12) Hydrolyse von Stärke: +
(13) Reduktion von Nitrat: +
(14) Produktion von Indol: -
(15) Verflüssigung von Gelatine: +
(16) Zersetzung von Casein: +
(17) Filmbildung auf einem flüssigen Medium: +
(18) Coagulation von Milch: -, und
(19) Peptonisierung von Milch: +

2. Verwendung des Bacillus subtilis C-3102 (FERM BP-1096) als Probiotikum für Tiere.

3. Futter, welches den Bacillus subtilis C-3102 (FERM BP-1096) nach Anspruch 1 enthält.

Revendications

1. Probiotique pour animaux qui comprend Bacillus subtilis C-3102 (FERM BP-1096) ayant les caractéristiques bactériologiques ci-dessous décrites:
(1) Grampositif
(2) Cellules: bâtonnets
(3) Spores: ovales
(4) Mobilité: positive
(5) Aérobie
(6) Catalase: +

(7) Croissance à 50 °C: +
(8) Croissance à pH 5.7: +
(9) Utilisation du citrate: +
(10) Production d'acides à partir de glucides
arabinose, glucose, xylose, mannitol: +
(11) Réaction VP: +
(12) Hydrolyse de l'amidon: +
(13) Réduction des nitrates: +
(14) Formation d'indole: -
(15) Liquéfaction de la gélatine: +
(16) Décomposition de la caséine: +
(17) Formation d'une pellicule à la surface d'une bouillon de culture liquide: +
(18) Caillement du lait: -
(19) Peptonification du lait: +

2. Emploi de Bacillus subtilis C-3102 (FERM BP-1096) comme probiotique pour animaux.

3. Aliments contenants Bacillus subtilis C-3102 (FERM Bp-1096) défini dans la sollicitation 1.